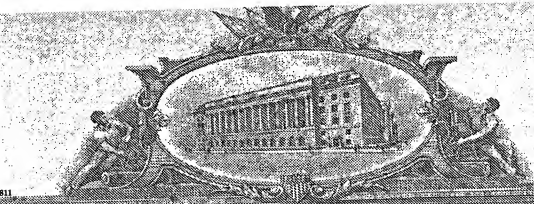

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APPLICATION NUMBER: *60/499,634*
FILING DATE: *August 28, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/28000*

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

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INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Chad	KENNEDY	Highley, Arizona, USA
Gholamreza	EHTESHAMI	Scottsdale, Arizona, USA

Additional inventors are being named on the 1 *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

HYDROGELS FOR MODULATING CELL MIGRATION AND MATRIX PRODUCTION

CORRESPONDENCE ADDRESS

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 9

☐ CD(s), Number

☐ Drawing(s) *Number of Sheets*

☑ Other (specify) Return Postcard

☐ Application Data Sheet. See 37 CFR 1.76

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☑ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees

☑ The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 17-0055

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Respectfully submitted,

SIGNATURE Kittie A. Murray

TYPED or PRINTED NAME Kittie A. Murray

TELEPHONE (602) 229-5200

Date 08/28/03

REGISTRATION NO. *(if appropriate)* 30,346

Docket Number: 130588.00051

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☑ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 80.00

Complete if Known	
Application Number	
Filing Date	August 28, 2003
First Named Inventor	Chad Kennedy
Examiner Name	
Group Art Unit	
Attorney Docket No.	130588.00051

METHOD OF PAYMENT *(check all that apply)*

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FEE CALCULATION

1. BASIC FILING FEE

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Independent Claims	-3** =	X		=0.00
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1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
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1812	2,520	1812	2,520	For filing a request for ex parte reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	410	2252	205	Extension for reply within second month	
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1254	1,450	2254	725	Extension for reply within fourth month	
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1402	320	2402	160	Filing a brief in support of an appeal	
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1452	110	2452	55	Petition to revive - unavoidable	
1453	1,300	2453	650	Petition to revive - unintentional	
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1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	750	2809	375	Filing a submission after final rejection (37 CFR § 1.129(a))	
1810	750	2810	375	For each additional invention to be examined (37 CFR § 1.129(b))	
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1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

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Name (Print/Type)	Kittie A. Murray	Registration No. (Attorney/Agent)	30,288	Telephone	(602) 229-5200
Signature	Kittie A. Murray			Date	August 28, 2003

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Applicant: Kennedy *et al.*

Filed: August 28, 2003

Title: HYDROGELS FOR MODULATING CELL MIGRATION AND MATRIX PRODUCTION

Docket No.: 130588.00051

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INVENTOR(S)/APPLICANT(S)		
Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
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Stephen	MASSIA	Mesa, Arizona, USA

Number 2 of 2

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PATENT

PROVISIONAL APPLICATION

of

CHAD KENNEDY
GHOLAMREZA EHTESHAMI
DORIS HOM
STEPHEN MASSIA

For

UNITED STATES LETTERS PATENT

on

HYDROGELS FOR MODULATING CELL MIGRATION AND MATRIX PRODUCTION

Attorneys:
QUARLES & BRADY STREICH LANG L.L.P.
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Hydrogel for Modulating Cell Migration and Matrix Deposition

Field of the Invention

The present invention, a hydrogel consisting of a pre-gel conjugate, is intended for use in the field of medicine. Specifically, the pre-gel is comprised of dextran, peptide sequences susceptible to proteolytic cleavage and peptide or protein therapeutics. The incorporation of a cleavable peptide into a biodegradable, three-dimensional polysaccharide network regulates tissue growth by providing degradation sites for cell-released proteases. These components of the hydrogel are suited to modulate cell migration and invasion. Moreover, the pre-gel conjugate has high biocompatibility suited for use as an injectable gel following polymerization (gel formation) or polymerization in situ. Once injected, the gel can serve as a modulator to cell growth and protein synthesis, which has broad medical applications such as, tissue engineering, wound healing, cancer, limiting angiogenesis and cardiovascular disease. Addition of specific therapeutics in the gel, yields an ideal local drug delivery vehicle. Research applications include in vitro diagnostic studies of local peptide or protein effects on cell migration, invasion, proliferation, protein expression, cytotoxicity, DNA and mRNA expression, etc. The gel form could be used in diverse conditions from microarrays and biosensors to medical implants.

Background of the Invention

Hydrogels have been gaining significance as an effective biomaterial; its interactions with biological tissues or functions demonstrate high biocompatibility. Specifically, a hydrogel is a hydrophilic polymer network that can store a percentage of water [1]. This is created by forming crosslinks between polymeric strands, which can be chemically modified to react using light, heat, or pH changes. Dextran hydrogels can be prepared in this manner [2].

Of the many possible biocompatible gels, dextran gels have been recognized as a good material for experimental use and FDA approved. As a natural polymer, dextran is a polysaccharide that is biodegradable through hydrolysis or enzymatic reactions [2]. Additionally, their hydroxyl functional groups allow for chemical modification to either form cross-links in hydrogel formation or attachments to molecules [3]. As a material, dextran is inexpensive and easy to manipulate. This invention created a new kind of dextran hydrogel suited for controlled growth of tissue by adding a proteolytically cleavable peptide [4].

Degradation of hydrogels depends on hydrolysis of either the cross-links or the polymer backbone. This process is unique to the different hydrogel compositions. Some may be synthesized with targeted degradation of the cross-links or the backbone. Regular degradation of polymers in general relies on adsorption of medium on the polymer surface, diffusion of medium into the polymer, chemical reactions, diffusion of degradation products, and desorption of degradation products [5]. For the most part, degradation is mitigated by specific enzymatic hydrolysis of hydrogel components.

Meyvis et al. incorporated (endo-) dextranase during cross-linking of dextran methacrylated hydrogels in order to introduce degradable units in the polymer [6]. The degradation rate would depend on the cross-link density and the amount of dextranase present. It was shown that lower cross-link density correlated with shorter degradation times. A similar method was employed by O. Franssen et al. to produce degradation-controlled release of proteins [7]. Enzymatically-degrading dextran hydrogels were made by co-entrapment of dextranase. In addition, chemically degrading gels of hydroxyethyl methacrylated dextran were obtained to demonstrate degradation time with cross-linking. Mann and West et al. produced proteolytically degradable domains within hydrogels to promote degradation of the cross-links [4].

The introduction of the cleavable peptide is intended to modulate the migration of cells. Cell migration is mitigated by response to extracellular signals and phenotypic preference; this concept is an established notion in cell biology. Through chemotaxis, the cells move in a direction controlled by a gradient of diffusible chemicals [8]. In cases of injury, this effect is brought about by signaling from the immune

system. For vascular injury following angioplasty, the response of smooth muscle cells to injury signals leads to accumulation of neointimal smooth muscle cells. Cell migration studies are typically conducted in experimental assays with modified Boyden chambers. These chambers have inserts that separate cells from chemoattractants in the lower well. The chemical signaling causes the migration of cells through micron size pores; these pore sizes and density may be specific for each type of cell.

The movement of cells to the extracellular matrix is facilitated by the release of matrix metalloproteinases (MMPs) to dissolve numerous pericellular substrates [8]. These extracellular proteinases are able to degrade structural proteins of the extracellular matrix (ECM), cleave cell surface molecules, and other proteins. This regulates cell behavior in response to physiological processes including embryonic development, wound repair, cancer, and tissue morphogenesis [8]. Since their identification, several classes of MMPs have been discovered to function in different ways; some specifically target amino acid residues to remove barriers to invasion. Tissue inhibitors of metalloproteases (TIMPs) are naturally occurring inhibitors of MMPs [9]. Four known TIMP isoforms, TIMP-1, -2, -3, and -4, have been studied for their binding affinity to different MMPs. TIMPs inhibit MMP activity by binding to them, producing an inactive 1:1 complex [10]. By targeting MMP activity, tissue regulation may be possible.

Summary of the Invention

The dextran hydrogel was synthesized and tested in a combination of steps. Cell migration assays were used to validate the concept. The peptide was synthesized by the ASU Protein Chemistry Facility by Dr. Daniel Brune and Dr. John Lopez. Polypeptides were synthesized on Fmoc-XAL-PEG-polystyrene resin on a Miligen-Biosearch 9050 peptide synthesizer using standard Fmoc chemistry with HATU (*N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-b]pyridino-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide, Applied Biosystems) as the activator. After synthesis, peptides were cleaved from the synthesis resin with trifluoroacetic acid containing 2.5% triisopropyl silane and 2.5% ethanedithiol as scavengers. The peptides were then precipitated and washed 3 times with diethyl ether and vacuum dried overnight. The molecular masses of the peptides were confirmed using mass spectroscopy. The final sequence synthesized is Cys-Gly-Gly-Leu-Gly-Pro-Ala-Gly-Gly-Lys-Gly (CGGLGPAGGKG).

To synthesize the conjugated dextran, oxidized dextran (20% oxidized) is reacted with peptide in phosphate buffer. For a yield of 100 mg of conjugated dextran, 0.1 g peptide is reacted with 0.1 g dextran; this determination results from an approximated ratio. It is assumed that the molecular weight of dextran is 40 kD and the peptide is 872 MW. The two reactants are mixed well in 2 mL of phosphate buffer. The reaction is allowed for 48 hours. Six hours prior to completion, 0.02 g $NaBH_4$ is added to reduce possible disulfide bond formations between the peptides. To purify the product, the entire solution is placed in a dialysis bag (Snakeskin Pleated Dialysis Tubing Product, 10,000 MW cut-off). Dialysis is run for two days with the water changed every few hours to facilitate the diffusion of unreacted elements.

To synthesize the hydrogel, the conjugated dextran is reacted with acryloylated dextran (degree of substitution is 20%) in phosphate buffered saline (Gibco, without calcium chloride and magnesium chloride). A photo initiator is prepared through dissolution of 300 mg 2,2-dimethoxy-2-phenyl-acetophenone (Aldrich 19,611-8) in 1 mL of N-vinylpyrrolidone (Aldrich V340-9). An ultraviolet ray lamp (B-100AP Ultra-Violet Products, Inc., Upland, CA) of peak wavelength 370 nm was used to initiate cross-linking between the conjugated dextran and acryloylated dextran.

To validate the effectiveness of the hydrogel as a barrier, a cell migration assay was conducted. Bovine endothelial cells (CRL GMC 7372B, NIGMS Human Genetic Cell Repository) were maintained in minimum essential medium (MEM) (Gibco, 10370-021) supplemented with 10% FBS (Gemini-Bioproducts, 100-106), 1% antibiotic-antimycotic (Gibco, 15240-062), and 1% L-glutamine (Gibco, 25030081) in 5% CO_2. The cell migration assay was conducted on a 24-well plate (BD Falcon™ HTS Fluoroblok Multiwell Insert System, 8 μm pore size) with a light-opaque membrane of PET. The plates were first pre-wetted with 0.5 mL of 1% triton X-100 in PBS in order to improve the affinity of the polystyrene sides of the insert to the hydrogel. The different ratios of dextran and photo initiator were

dissolved in PBS at the 20 wt%. To produce a 1 mm thick gel, 30 μL of the gel mixture is pipetted into each well.

Preparation of the cells for use involves exposing cells to a 3 mL solution containing fluorescent probe (CellTracker™ Probes by Molecular Probes, C-2925, C-7025). The cells are incubated for 30 minutes and the working solution is replaced with fresh media afterwards. Preparation of the cells for use involves exposing cells to a 3 mL solution containing fluorescent probe (CellTracker™ Probes by Molecular Probes, C-2925, C-7025). The cells are incubated for an additional 30 minutes. Cells are detached using 0.25% trypsin, 1% EDTA, pelleted, counted and resuspended in serum free media at a concentration of 1×10^6 cells/mL. The cells are seeded on top of the hydrogels in a volume of 300 μL. The lower wells are filled with 1000 μL of supplemented media via the access ports.

Endothelial cell migration is quantified by fluorescent microscopy at 4 hours, 24 hours, and 48 hours. Three randomized pictures of each well will be taken and stored for image analysis. Using National Instruments IMAQ Vision Builder 6, the percentage of migrated cells is determined as the average of three images. The cell area is represented as the percentage of the image area. ANOVA techniques are performed using a $\alpha=0.05$ to determine significant difference between hydrogel ratios.

Details

The invention is further described with reference to Appendix A: "Dextran-Based Hydrogel to Regulate Tissue Growth" by Hom, D.C., Kennedy, Chad E., Ehteshami, G. and Massia, S.P., the entire contents of which are hereby incorporated by reference.

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[10] Gomis-Ruth, F.X. et al. Mechanisms of Inhibition of the Human Matrix Metalloproteinase Stromelysin-1 by TIMP-1. *Nature*. 1997. 389: 77-81.

APPENDIX A

Dextran-Based Hydrogel to Regulate Tissue Growth

D.C. Hom, Chad E. Kennedy, G. Ehteshami, S.P. Massia[1]
Harrington Department of Bioengineering, Arizona State University, AZ, USA[1]

Abstract—**A dextran hydrogel was synthesized from cross-linking a blend of acryloylated dextran and dextran bioconjugate. The conjugated dextran contains a matrix metalloproteinase (MMP) sensitive peptides forming crosslinks that promotes cell-induced enzymatic degradation of the hydrogel and migration through the gel. By changing the ratio of cleavable cross-links to stable cross-links in the hydrogel, the extent of cell invasion into the gel (tissue ingrowth) can be regulated. At twenty-four hours, the extent of migration ranged from 0.25 to 13.6% area covered by cells based on the level of degradable crosslinks within the gel. It was discovered that the gel proportion containing 80% acryloylated dextran and 20% conjugated dextran limited the most cell migration.**

Keywords—**Cell migration, dextran**

I. Introduction

Vascular complications create the need for improved treatment methods in percutaneous transluminal coronary angioplasty (PCTA). These complications include stent thrombosis, in-stent restenosis, and intimal hyperplasia [1-2]. Stent coatings of hydrogels which regulate tissue growth may prolong initial therapeutic benefit gained from the procedure. Modification of existing stent technology provides opportunities for direct interaction with the body's healing responses. This characteristic, combined with established understanding of stent deployment, can produce a device better suited for treating cardiovascular disease.

Of the many possible biocompatible gels, dextran gels have been recognized as a good material for experimental use. As a natural polymer, dextran is a polysaccharide that is biodegradable through hydrolysis or enzymatic reactions [3]. Additionally, their hydroxyl functional groups allow for chemical modification to either form cross-links in hydrogel formation or attachments to molecules [4-5]. As a material, dextran is inexpensive and easy to manipulate. The goal of this study is to create a new kind of dextran hydrogel suited for controlled growth of tissue by adding a degradable peptide [6].

II. Methodology

The dextran hydrogel was synthesized and tested in a combination of steps. Cell migration assays were used to validate the concept.

1) *Synthesis of peptide*: The peptide was synthesized by the ASU Protein Chemistry Facility by Dr. Daniel Brune and Dr. John Lopez. Polypeptides were synthesized on Fmoc-XAL-PEG-polystyrene resin on a Miligen-Biosearch 9050 peptide synthesizer using standard Fmoc chemistry with HATU (*N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-b]pyridino-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide, Applied Biosystems) as the activator. After synthesis, peptides were cleaved from the synthesis resin with trifluoroacetic acid containing 2.5% triisopropyl silane and 2.5% ethanedithiol as scavengers. The peptides were then precipitated and washed 3 times with diethyl ether and vacuum dried overnight. The molecular masses of the peptides were confirmed using mass spectroscopy. The final sequence synthesized is Cys-Gly-Gly-Leu-Gly-Pro-Ala-Gly-Gly-Lys-Gly (CGGLGPAGGKG).

2) *Synthesis of conjugated dextran*: Oxidized dextran (20% oxidized) is reacted with peptide in phosphate buffer. For a yield of 100 mg of conjugated dextran, 0.1 g peptide is reacted with 0.1 g dextran; this determination results from an approximated ratio. It is assumed that the molecular weight of dextran is 40 kD and the peptide is 872 MW. The two reactants are mixed well in 2 mL of phosphate buffer. The reaction is allowed for 48 hours. Six hours prior to completion, 0.02 g $NaBH_4$ is added to reduce possible disulfide bond formations between the peptides.

To purify the product, the entire solution is placed in a dialysis bag (SnakeSkin Pleated Dialysis Tubing Product, 10,000 MW cut-off). Dialysis is run for two days with the water changed every few hours to facilitate the diffusion of unreacted elements.

3) *Synthesis of dextran hydrogel*: The conjugated dextran is reacted with acryloylated dextran (degree of substitution is 20%) in phosphate buffered saline (Gibco, without calcium chloride and magnesium chloride). A photoinitiator is prepared through dissolution of 300 mg 2,2-dimethoxy-2-phenyl-acetophenone (Aldrich 19,611-8) in 1 mL of N-vinylpyrrolidone (Aldrich V340-9). An ultraviolet ray lamp (B-100AP Ultra-Violet Products, Inc., Upland, CA) of peak wavelength 370 nm was used to initiate cross-linking between the conjugated dextran and acryloylated dextran.

4) *Cell migration assay*: Bovine endothelial cells (CRL GMC 7372B, NIGMS Human Genetic Cell Repository) are maintained in minimum essential medium (MEM) (Gibco, 10370-021) supplemented with 10% FBS (Gemini-Bioproducts, 100-106), 1% antibiotic-antimycotic (Gibco, 15240-062), and 1% L-glutamine (Gibco, 25030081) in 5% CO_2.

The cell migration assay was conducted on a 24-well plate (BD Falcon™ HTS Fluoroblock Multiwell Insert System, 8 μm pore size) with a light-opaque membrane of PET. The plates were first pre-wetted with 0.5 mL of 1% triton X-100 in PBS in order to improve the affinity of the polystyrene sides of the insert to the hydrogel. The different ratios of dextran and photoinitiator were dissolved in PBS at the 20 wt%. To produce a 1 mm thick gel, 30 μL of the gel mixture is pipetted into each well.

Preparation of the cells for use involves exposing cells to a 3 mL solution containing fluorescent probe (CellTracker™ Probes by Molecular Probes, C-2925, C-7025). The cells are incubated for 30 minutes and the working solution is replaced with fresh media afterwards. Preparation of the cells for use involves exposing cells to a 3 mL solution containing fluorescent probe (CellTracker™ Probes by Molecular Probes, C-2925, C-7025). The cells are incubated for an additional 30 minutes. Cells are detached using 0.25% trypsin, 1% EDTA, pelleted, counted and resuspended in serum free media at a concentration of 1 x 10^6 cells/mL. The cells are seeded on top of the hydrogels in a volume of 300 μL. The lower wells are filled with 1000 μL of supplemented media via the access ports.

Using National Instruments IMAQ Vision Builder 6, the percentage of migrated cells is determined as the average of three images. The cell area is represented as the percentage of the image area. ANOVA techniques will be performed using an α=0.05 to determine significant difference between hydrogel ratios.

III. Results

A. Synthesis of dextran hydrogel

Cross-linking of the conjugated dextran and acryloylated dextran produced white, opaque gels after exposure to UV for a few minutes. The pre-gel mixture remains a viscous liquid without addition of the photoinitiator.

B. Cell Migration Assay

The cell migration assay produced data with a noticeable trend to reduced cell migration with higher % of acryloylated dextran in relation to conjugated dextran. This is seen in Fig. 1. The data is presented as % image area covered by cells at three different timepoints. A-dex refers to acryloylated dextran and conjugated refers to conjugated dextran.

At four hours, there does not seem to be great difference between the mean values. Additionally, the variances appear large. There was no significant difference between the means using ANOVA for all the tested ratios. Significance was also found between the means for the 20% acryloylated dextran and 80% conjugated dextran blend and

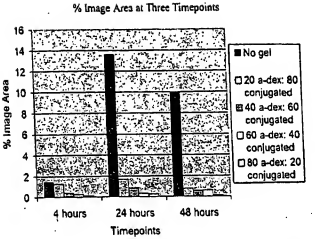

Fig. 1. Percentage of cells covering the image area observed at 4, 24, and 48 hours. A-dex refers to acryloylated dextran and conjugated refers to conjugated dextran. The ratios are given in weight percentages.

the 60% acryloylated dextran and 40% conjugated dextran blend. The P-value, 0.033 is also less than the acceptable value of 0.05; this supports the assumption of significance.

The data at 24 hours are similar to the trend in the four hour results; however, there was found to be significance between all the means (P = 0.00). At this time, the percentage of cells migrating through the membrane without the gel barrier increased significantly. Significance was found between the 20% acryloylated dextran and 80% conjugated dextran blend and 80% acryloylated dextran and 20% conjugated dextran blend (P = 0.00).

At 48 hours, the same trend of decreasing cell migration with increasing acryloylated dextran is seen. However, statistically, the 40% acryloylated dextran and 60% conjugated dextran blend and 60% acryloylated dextran and 40% conjugated dextran blend ratios appear to be similar. The significance found between the 20% acryloylated dextran and 80% conjugated dextran blend and 80% acryloylated dextran and 20% conjugated dextran blend (P = 0.00) still held. At forty-eight hours, however, the percentage values seemed to decrease. This may be attributed in part to the loss of the dye through cell divisions.

IV. Discussion

For the data taken at the four, twenty-four, and forty-eight hour time points, a general trend of cell migration can be noted. For comparison between means of two ratios, significant results can be determined. At the four hour mark, the differences between the ratios appear slight. Graphically, cell migration appears to decrease as the acryloylated dextran increases. The acryloylated dextran does not have the cleavable sites to allow cell migration. Expectedly, the number of cells going through the gel

should decrease as more of these sites are replaced by MMP stable cross-links. Fig. 1 shows cell migration range from 0.09 to 1.2% of the total image ratio and 1.5% for the wells without a gel. Although the differences in the means at the two extreme ratio values are significant, the results at this time point may be affected by cell response to initial seeding. Later time points revealed a more dramatic difference in cell migration.

At twenty-four hours, the amount of cell migration in the well without gel increased greatly. Without a gel as a barrier to the chemoattractants, the cells migrated at a much faster rate than the wells with a gel barrier. The range for the gel inserts increased to a 0.25 to 1.7%. The wells without gel increased to 13.6%. This data showed the most significant difference between the means for almost every analyzed combination. The difference between the two extremes (20 a-dex: 80 conjugated and 80 a-dex: 20 conjugated) is maintained, but other combinations showed significance also. Between the 20 a-dex: 80 conjugated and 40 a-dex: 60 conjugated ratios, the mean was found to be statistically different. At twenty-four hours, a greater cellular response is noted by increase in cells that came through the pores. The percentages did not seem to increase dramatically. For the highest proportion of conjugated dextran, cells migrated the most; however, the result does not seem to show much increase from the previous result taken at four hours. Looking at Fig. 1, the percentage of cells migrating through the gel did not increase greatly for each ratio. If a migration amount at less than 1% is desired, the three ratios, 40 a-dex: 60 conjugated, 60 a-dex: 40 conjugated, and 80 a-dex: 20 conjugated seem to fulfill this specification.

At forty-eight hours, cell migration seemed to decrease according to image analysis. The expected result would be increased cell migration. The range in cell migration in the inserts ranged form 0.1% to 0.7%, while the inserts without gel had 9.9% recorded. This deviation could be attributed to image analysis (threshold setting for cell count) or in the performance of the dye after two days. The concentration of cells (30,000 cells per 30 μL) has not been found to be problematic in maintaining cell growth; however, in the division of cells, the intensity of the dye to be measured may be partitioned. Despite this, the general trend in decreased cell migration continues to hold. Statistical difference was found between the extreme ratio combinations again and between 40 a-dex: 60 conjugated and 80 a-dex: 20 conjugated. The use of conjugated dextran to allow cell migration appears to produce differential cell migration as expected.

The experiment was not conducted after forty-eight hours due to limited cell migration. The supplemented media would not maintain cell growth without replenishment after a couple of days. Also, the chemical gradient created by the 10% serum found in the bottom of the cells may have equilibrated at this point. Without a force driving cell migration, no dramatic changes in invasion will be documented. This point may have affected the values at forty-eight hours.

The statistical analysis using ANOVA helped show which means in cell migration differed. A general analysis over the four ratios can determine whether the means differ; however, the separate comparisons over specific ratios would determine the significance between two. The 20 a-dex: 80 conjugated and 80 a-dex: 20 conjugated comparisons were always found to be significantly different. This result shows that cell migration is regulated by the incorporation of the cleavable peptide into the gel structure. Also, without a gel as a barrier, the cells showed increase migration. These analyses show the effectiveness of the gel as a barrier to migration and can show varying response to varying chemical composition of the hydrogel.

V. Conclusion

The following conclusions were drawn from this investigation:

- The amount of cell migration can be controlled by the number of cleavable sites introduced in a dextran hydrogel.
- Dextran ratios with higher proportions of acryloylated dextran limited the most cell migration.

For the different kinds of gels prepared from ratios of conjugated dextran and acryloylated dextran, the amount of cell migration was shown to be varied at three different time points. Although the means were not significant between all ratios among all three time points, significance was found between the ratios with greater proportions of conjugated dextran and acryloylated dextran. It appears that the peptide in conjugation with the dextran regulated the amount of cell migration through the hydrogel.

The results from this initial experiment demonstrated the possibility of controlling cell migration using a dextran hydrogel as a barrier. For therapeutic use, *in vivo* tests for biocompatibility need to be pursued to warrant medical application.

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We claim:

1. A bioconjugate comprising dextran and a proteolytically cleavable peptide.

2. A drug delivery device comprising a bioconjugate of Claim 1 and a therapeutic drug.

3. A method of controlling migration of cells on a surface comprising contacting said cells with the bioconjugate of Claim 1.

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